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ENCAPSULATION OF VITAMIN D IN THE EXINE-ALGINATE-CHITOSAN MICROCAPSULE SYSTEM

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Highlights

- Exine microcapsules are obtained from *C. libani* pollen using a microwave-assisted chemical process.
- Microwaved exine microcapsules remain structurally intact.
- Vitamins D_2 and D_3 could be loaded in exine microcapsules using ethanol.
- Alginate and chitosan are capable of effectively stabilising D_2 and D_3 loaded-exine microcapsules.
- Vitamins D_2 and D_3 release depends on time and temperature in the microcapsule system.

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ABSTRACT: The insufficiency of vitamin D, resulting from inadequate exposure to sunlight and/or insufficient dietary intake, remains a major public health concern on a global scale. In this study, vitamin D_2 and D_3 were microencapsulated using sporopollenin exine microcapsules extracted from *Cedrus libani* pollens. After loading vitamin D into the microcapsules, they were coated with chitosan, an edible, biocompatible, and mucoadhesive polysaccharide, and alginate (a food additive agent coded E401). Exine microcapsules were extracted by microwave irradiation-assisted chemical method, and structural and morphological examination of exine structures was performed by FT-IR, TGA, SEM, and SEM-EDX analyses. After loading vitamin D into microcapsules in an ethanol medium, the loaded microcapsules were immobilised into the alginate matrix in a calcium chloride solution. D_2 and D_3 were loaded into 100 mg of sporopollenin exine microcapsules, resulting in loading efficiencies of 31.5 mg and 16.0 mg, respectively. The vitamin D release performance of the microcapsules was examined depending on time and temperature after they were coated with a thin chitosan layer. The release of the highest amount of vitamin D_2 and D_3 occurred at a temperature of 37°C. Encapsulating vitamin D molecules in chitosan and alginate creates a barrier against degrading environmental conditions, which helps prevent the loss of vitamin D biological activity. This can improve vitamin D dietary supplements' storage, preservation, and marketing requirements.

Keywords: *Alginate, Cedrus libani, Chitosan, Encapsulation, Sporopollenin, Vitamin D*

1. INTRODUCTION

Vitamin D is a fat-soluble vitamin that can be found in both plants and animals. It comes in two forms, ergocalciferol (D_2) in plants and cholecalciferol (D_3) in animals [1-3]. Vitamin D is crucial in many bodily functions such as calcium metabolism, bone health, and cellular and metabolic cycles [4].

Vitamin D deficiency is common due to its low presence in foods and resistance to heat and cooking. For this reason, it is essential to enrich foods with vitamin D and make supplements available [5]. Microencapsulation processes enhance vitamin D's bioavailability, as it is easily absorbed in the intestine and suitable for food preservation and processing [6]. Since the chemical molecules used during microencapsulation may cause health problems when taken into the body, it has been observed that more natural molecules can be preferred in the processes.

Plant pollen's exine shells are ideal microcarriers for drug delivery [7]. Sporopollenin is a substance on the outer surface of spores and pollen grains. It is an abundant and edible material with a porous morphology and can maintain its structural integrity during extraction. Due to its thermal stability, sporopollenin grains, also known as exine capsules, are resistant to chemical and biological attacks [8]. It is challenging to microencapsulate sporopollenin microcapsules due to their complex microstructure and poor solubility [9].

Alginate has a unique property of selectively binding with multivalent cations to form a gel, and $CaCl_2$ is commonly used to create calcium alginate gel. Sodium alginate ($NaC_6H_7O_6$) is a linear polysaccharide derivative of alginic acid composed of 1,4-β-d-mannuronic and α-1,4-guluronic acids. Alginates have the unique property of turning into a gel form when dissolved in water. This transformation occurs almost instantaneously by replacing monovalent ions (e.g., Na^+) with divalent

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ions (specifically, Ca^{2+}), leading to the formation of a gel structure from a low-viscosity solution. The resulting gel is a copolymer composed of two different monomer units [10]. Alginate is a type of biocompatible polymer that is degradable and has the advantage of being low-cost [10, 11]. It is a non-toxic component that protects the upper gastrointestinal mucous membranes [10]. The dry alginate can absorb water and release the drug in a controlled manner. Studies indicate high compatibility between core and shell materials in alginate encapsulations [12]. However, alginate capsules alone are insufficient for successful encapsulation [10].

Chitosan is a copolymer produced by partially or entirely deacetylating chitin under alkaline conditions with either sodium hydroxide or chitin deacetylase enzyme [13, 14]. Chitosan is a commonly used excipient in the pharmaceutical industry for direct tablet compression and as a tablet integrator for producing controlled-release dosage forms. This is due to its non-toxic, biocompatible, and biodegradable properties. In recent years, chitosan microspheres have been developed for site-specific drug delivery, including intestinal selective drugs, anticancer agents, and mucoadhesive drug delivery systems [15].

Studies have been conducted on microencapsulation of Vitamin D, with one study using Vitamin D_2 as a model drug. The study found that using spray drying, the chitosan micronuclei efficiently trapped the Vitamin D_2. The microcapsules were then coated with ethylcellulose. Tests on the morphology and release properties of the microcapsules were carried out. *In vitro* release results showed that the microcapsules could achieve sustained release in the intestinal environment [15]. A recent study involved the synthesis of a new amphiphilic chitosan derivative of N, N-dimethyl hexadecyl carboxymethyl chitosan, followed by the loading of vitamin D_3. The study found that the vitamin was loaded at a rate of 53.2%. The *in vitro* release process of the loaded vitamin D_3 was initially rapid and then followed by a sustained release [16]. In another study, the process of encapsulating vitamin D by complex coacervation was studied. The encapsulation was done using a carbohydrate, specifically cress seed mucilage and gelatin protein. The study found that the efficiency and payload of encapsulation were significantly affected by the core-to-shell ratio and the mucilage/gelatin ratio. The best microcapsules had 67.93% and 50.9% efficiency and loading capacity, respectively [17].

This study was conducted to create a microencapsulated form of vitamin D using sporopollenin exine microcapsules extracted from *Cedrus libani* pollens. Both forms of vitamin D, D_2 and D_3, were loaded into the exine microcapsules, which were then coated with alginate (a food additive, E401) and chitosan, an edible and biocompatible polysaccharide that is also mucoadhesive. The release profile of vitamin D from the microencapsulation system was studied at different temperatures and durations.

2. MATERIAL AND METHODS

2.1. Materials

Harvesting of *C. libani* pollens: The pollen samples used in this study were collected from Selcuk University Alaeddin Keykubad Campus in October 2022 (coordinates of the pollen collection site: 38.025663N, 32.504256E) (Konya, Türkiye). To collect the pollen, the cones were first dried, and then the pollen was shaken off. The collected pollen was then sieved to remove dust or other unwanted particles.

Chemicals: Ergocalciferol (D_2) (95220-1G, ≥98.0, Sigma-Aldrich), cholecalciferol (D_3) (C9756-1G, ≥98.0, Sigma-Aldrich), hydrochloric acid (Sigma-Aldrich), sodium hydroxide (Merck), methanol (≥99.7, GC, Sigma-Aldrich), chloroform (CAS: 67-66-3, HPLC, Loba Chemie), calcium chloride (Merck), ethanol (Emsure, ACS, ISO, Reag. Ph Eur, absolute, Merck), chitosan (448877, medium molecular weight, Sigma-Aldrich), acetic acid (Merck) and sodium alginate (W201502-1KG, Sigma-Aldrich) were used in the study.

2.2. Extraction of Exin Microcapsules from *C. Libani* Pollens

Extraction of exin microcapsules from *C. libani* pollens involved a three-step process to remove minerals, proteins, and pigments from the pollens. Firstly, 1.0 g of pollen was treated with 4.0 M 20.0 mL HCl solution in a microwave oven at 400 watts for three minutes to demineralise. The samples were then filtered with a Whatman filter paper and washed with pure water until neutral pH. Secondly, deproteinisation was done by treating the samples with 4.0 M NaOH 20.0 mL solution in a microwave oven at 400 watts for three minutes. Once again, the samples were filtered with a Whatman filter paper and washed with pure water until neutral pH. Lastly, the acid and base-treated pollen samples were incubated at room temperature in chloroform/methanol/water solution (4:2:1 by volume) at 400 watts for three minutes to remove pigments. Finally, the sporopollenin samples were washed thoroughly with distilled water and left to dry at room temperature.

2.3. Loading of Vitamin D_2 Or D_3 Molecules into The Exine Microcapsules

For each encapsulation process, 100.0 mg of sporopollenin was mixed with 2.0 mL of ethanol and 40.0 mg of vitamin D_2 or D_3 solution (20 mg/mL) to form a homogeneous mixture, which was then loaded into exine microcapsules. The product was mixed on a vortex for about 10 minutes to help the vitamin penetrate through the porous wall of the microcapsules.

The loading efficiencies of vitamin D_2 and D_3 into exine microcapsules were calculated based on mass measurements. The microcapsules were weighed before and after the vitamin loading to calculate the loading efficiency. Three repetitions were made, and the average weight was calculated to obtain a result closer to reality.

2.4. Coating of Vitamin D-Loaded Exine Microcapsules with Alginate and Chitosan

The process began by stirring 2.5 grams of sodium alginate in 50 mL of pure water at room temperature for 30 minutes, with the mixer set at 1000 rpm. Next, vitamins D_2 and D_3 were added separately to the alginate solution and mixed for another 30 minutes to ensure that they were evenly distributed. The mixing was carried out in a closed container to minimise air exposure. Capsules were formed by transferring the mixture into a burette and dropping it into a solution containing 100 mL of water and 5 grams of $CaCl_2$. The mixture was added drop by drop to the solution containing calcium ions, resulting in the formation of spherical gel structures [10].

The microcapsules immersed in calcium chloride solution were filtered using Whatman filter paper, washed with pure water, and then dried at room temperature. Later, vitamin D-loaded sporopollenin exine alginate microcapsules were added to the chitosan solution (2.0 g chitosan in 100 mL 2% acetic acid solution) and left for an hour. Using a needle under a light microscope, the microcapsules were carefully separated to avoid sticking together. To avoid vitamin loss and preserve their structure, the microcapsules were washed with a solution prepared by dissolving 5.0 g $CaCl_2$ in 100 mL water. However, it was observed that the presence of $CaCl_2$ prevented the release of vitamins. Therefore, the vitamin D-loaded sporopollenin exine alginate microcapsules were dried at room temperature after being immersed in a chitosan solution. Finally, they were stored in glass bottles.

2.5. The Release Profiles of Vitamin D_2 and D_3-Exine-Alginate-Chitosan System

To carry out release tests, calibration graphs were initially created. To prepare these graphs, specific amounts of vitamin D_2 or D_3 were added to ethyl alcohol and mixed using a vortex. The mixtures were then subjected to absorbance measurements (at 265 nm for vitamin D_2 and D_3) on a UV-Vis spectrophotometer (Fig. 1), and calibration curves were drawn [15]. These curves were then used to calculate the releases of the microcapsules.

The release tests for the vitamin D-microcapsule system were performed at different temperatures

(4, 20, and 37°C). A mixture of 15.0 mg of vitamin D loaded-exine-alginate-chitosan microcapsules and 3.0 mL of ethyl alcohol was shaken at 100 rpm for 120 minutes. The release of vitamin D was recorded at different temperatures. To study the effects of duration on the release of vitamin D from the vitamin D loaded-exine-alginate-chitosan microcapsule system, the release tests were repeated at different durations. A mixture of 15.0 mg of vitamin D loaded-exine-alginate-chitosan microcapsules and 3.0 mL of ethyl alcohol was shaken at 100 rpm at 37°C.

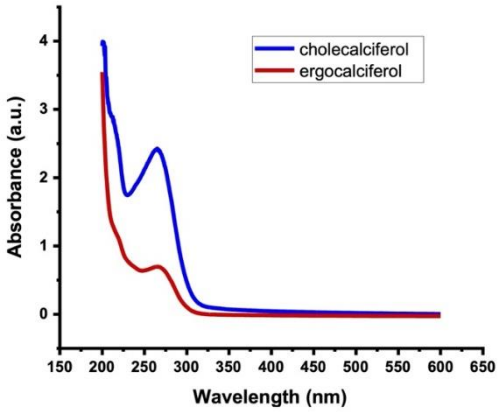

Figure 1. UV-vis spectra of ergocalciferol (D_2) and cholecalciferol (D_3).

3. RESULTS AND DISCUSSION

3.1. Extraction of Exine Microcapsules from *C. Libani* Pollens

Exine microcapsules were extracted using a microwave-assisted chemical method, and their structural and morphological characteristics were examined by FT-IR, TGA, SEM, and SEM-EDX analyses.

3.1.1. FT-IR Spectroscopy analysis

FT-IR spectra of untreated pollen were taken before and after the application of chemical treatments. The first spectrum was taken before any treatment, and the second spectrum was taken after the pollen grains were treated with acid. As a result of the acid treatment, there were noticeable differences and shifts in absorption band values in the pollen spectrum. An alkaline treatment was applied after the acid treatment, but no significant difference in the spectra was observed. This suggests that alkali treatment does not create any observable difference in the surface functional groups of the pollen. Finally, microwave irradiation was used to isolate exine structures in the last processing step by exposing the samples to a mixture of chloroform, methanol, and water. After this step, differences were observed in the FT-IR spectrum, as shown in Figure 2 (a–d).

The spectrum of *C. libani* pollen showed an intermolecular OH bond peak at 3359 cm^{-1}. Two peaks were recorded at 2920 and 2851 cm^{-1}, which are attributed to aliphatic C–H stretching vibrations. Furthermore, the absorption peak of –CNH bonds was observed at 2851 cm^{-1}, while the peak at 1112 cm^{-1} was due to C–O–C stretching vibration.

Compared with the spectrum of untreated *C. libani* pollen, changes in some peak values were observed in the spectrum of the extracted *C. libani* sporopollenin (Figure 1 d). Additionally, new peaks appeared in the spectrum of the exine (sporopollenin). The broad OH bond peak in the pollen spectrum shifted to 3365 cm^{-1}. The band at 1447 cm^{-1} can be associated with the C–O–H deformation vibration. The sharp peaks at 2922 and 2854 cm^{-1} can be interpreted as resulting from aliphatic C–H stretching of unsaturated fatty acids. The aliphatic C=C stretch band of sporopollenin structures was detected at 1604 cm^{-1} [18]. Literature comparisons show that structures isolated from *C. libani* pollen consist of sporopollenin [19].

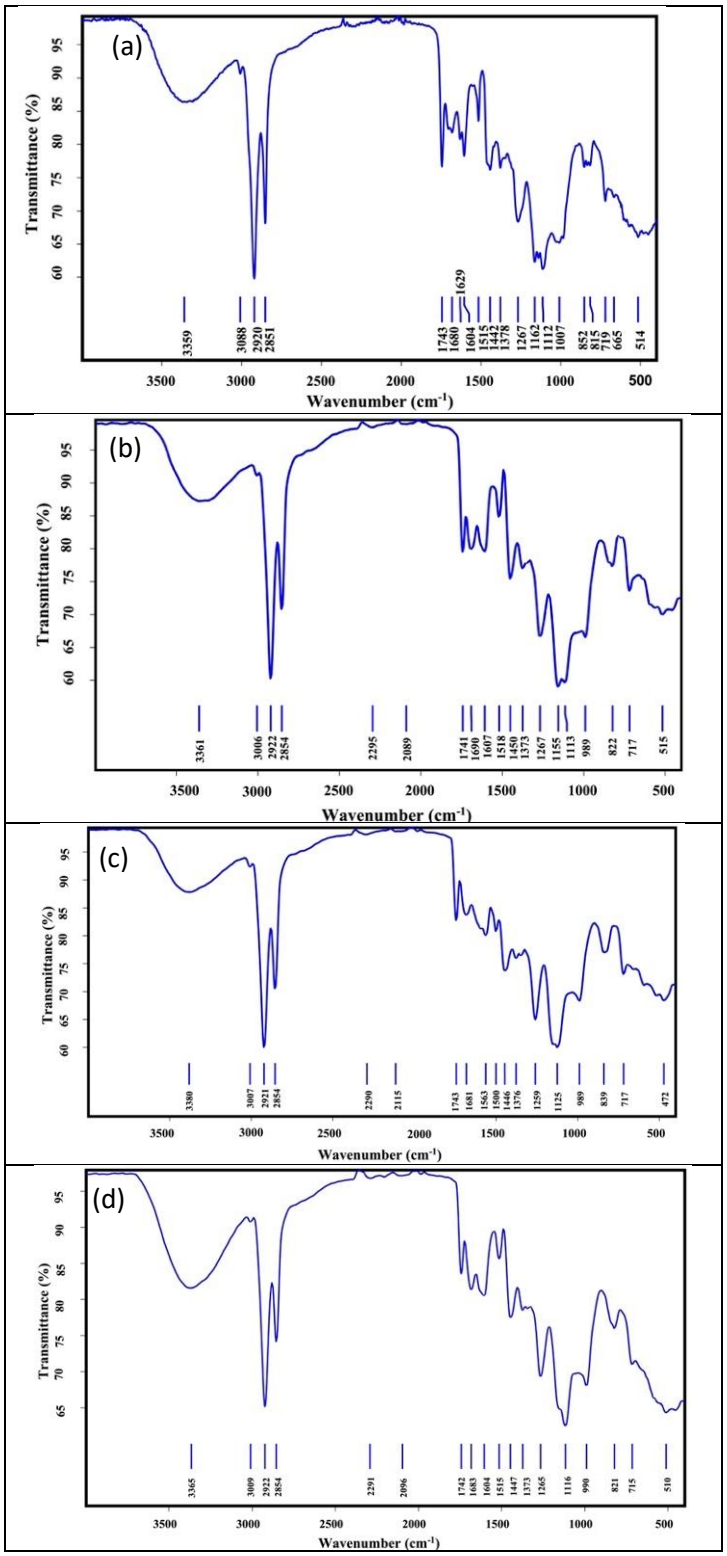

Figure 2. (a) FT-IR spectra of pristine *C. libani* pollens, (b) the pollens treated with a hydrochloric acid solution, (c) the pollens treated with a sodium hydroxide solution following the acid treatment, and (d) the pollens undergone the acid, the base, and the chloroform/methanol/water treatments (the exine microcapsules, the final product)

3.1.2. Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) was used to evaluate the thermal stability of both pollen and isolated exin capsules. The untreated pollen retained its mass up to around 250°C, after which mass loss occurred and continued until approximately 450°C. When treated with acid during exine isolation, the mass loss of the pollen began after 300°C. This suggests that pure pollen contains organic structures that can decompose at low temperatures, and the acid treatment removes these structures. After treatment with base and chloroform/methanol/water, mass loss began at higher temperatures due to the loss of an organic compound (Figure 3). Numerous studies have reported that exine microcapsules show higher thermal stability than the pollen from which they are extracted [7, 20]. Studies indicate that sporopollenin is the primary component of the exine structure. Sporopollenin is a highly stable polymer that is resistant to thermal, mechanical, chemical, and biological effects [9, 21].

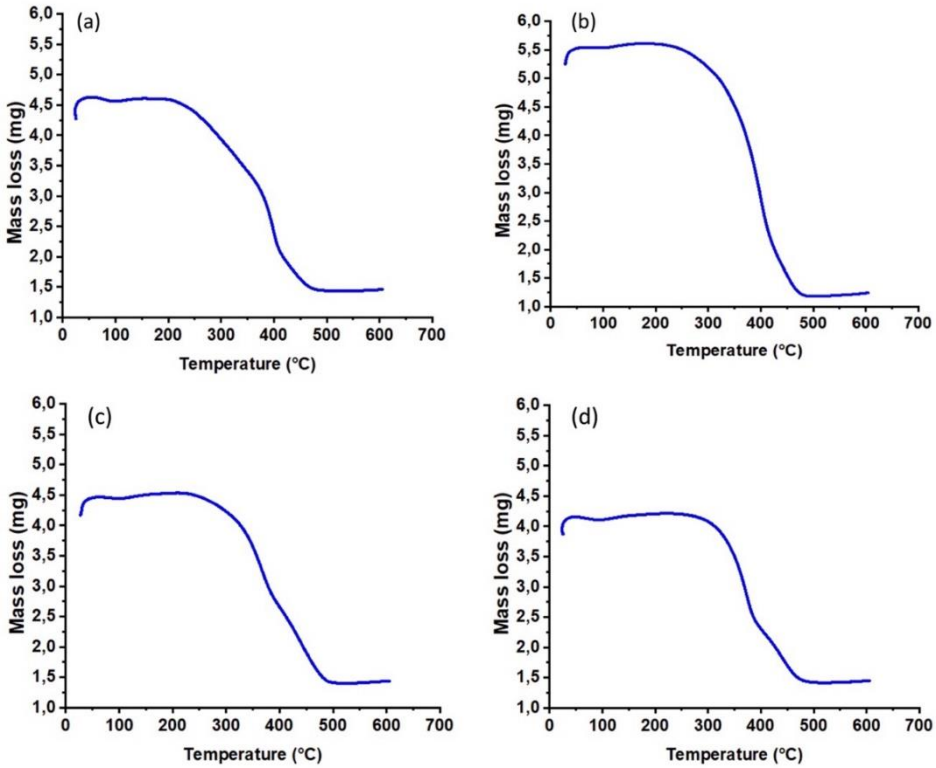

Figure 3. (a) TGA profiles of pristine *C. libani* pollens, (b) the pollens treated with a hydrochloric acid solution, (c) the pollens treated with a sodium hydroxide solution following the acid treatment, and (d) the pollens undergone the acid, the base, and the chloroform/methanol/water treatments (the exine microcapsules, the final product)

3.1.3. Analysis of the SEM images of the exine microcapsules

Pollens from *C. libani* were observed under a scanning electron microscope (SEM), along with the extracted exine structures (Figures 4 a–d). The aim was to examine the effect of a chemical extraction process on the pollen's morphological properties. To begin with, the pollen was treated with acid under microwave irradiation. This caused the removal of minerals and the formation of protrusions on the pollen's surface (Figure 4 b). Next, an alkaline treatment was applied, resulting in an increase in the number of protrusions on the pollen (Figure 4 c). This demonstrated that the alkaline treatment effectively removed protein-like structures within the pollen structure. Finally, a mixture of methanol, chloroform, and water was used to remove pigments and oily molecules from the completely empty

structure of the pollen (Figure 4 d).

Exine microcapsules were extracted from *C. libani* pollens using a modified version of chemical processes reported in the literature. The microcapsules were then emptied, and their structural integrity was preserved, so they could be loaded with vitamins for the study [7, 20]. It is noteworthy that no external heat source was utilised in the procedures outlined in the study. Rather, the experiment was conducted in a microwave oven at an energy level of 400W, with exposure to microwave radiation for a duration of 3 minutes. The study revealed that microwave radiation could significantly reduce the length of the extraction procedures, as opposed to the lengthy processes documented in previous literature.

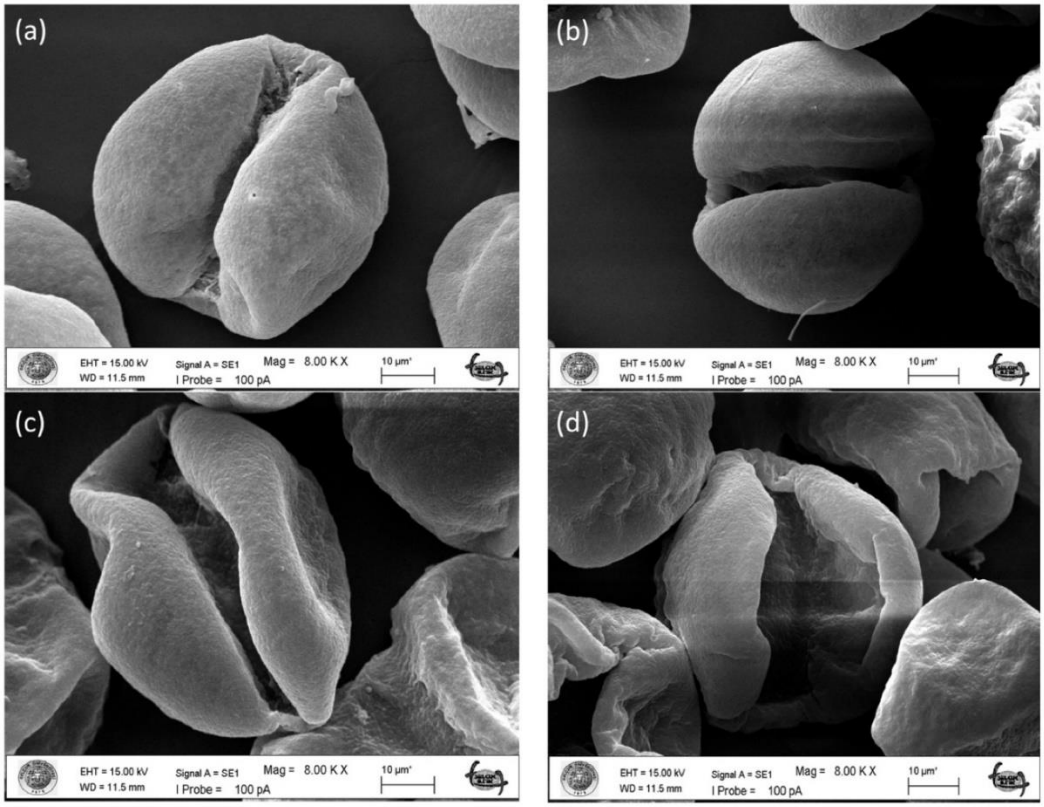

Figure 4. (a) SEM images of pristine *C. libani* pollens, (b) the pollens treated with a hydrochloric acid solution, (c) the pollens treated with a sodium hydroxide solution following the acid treatment, and (d) the pollens undergone the acid, the base, and the chloroform/methanol/water treatments (the exine microcapsules, the final product)

3.1.4. Analysis of the SEM-EDX profiles of vitamin D-exine-alginate-chitosan microcapsule system

The surface chemical compositions of microcapsules containing vitamin D, exine, chitosan, and alginate were analysed using SEM-EDX (Figure 5). The analysis revealed that microcapsules coated only with alginate (vitamin D-exine-alginate microcapsule) contained O, Ca, Cl, C, and Na. They had a higher mass of O, as presented in Tables 1 and 3. Na content was from alginate since the sodium salt of alginate was used in the study. The Ca and Cl contents were from the calcium chloride solution that was used to form alginate gel beads. After being coated with chitosan, the microcapsules (vitamin D-exine-chitosan-alginate microcapsule) contained N. Since sporopollenin, the material that forms the exine microcapsules doesn't contain N but C and O [22], the presence of N detected is due to chitosan, as presented in Tables 2 and 4. The SEM-EDX analysis results demonstrate that exine-alginate microcapsules with vitamin D_2 or D_3 are coated with chitosan.

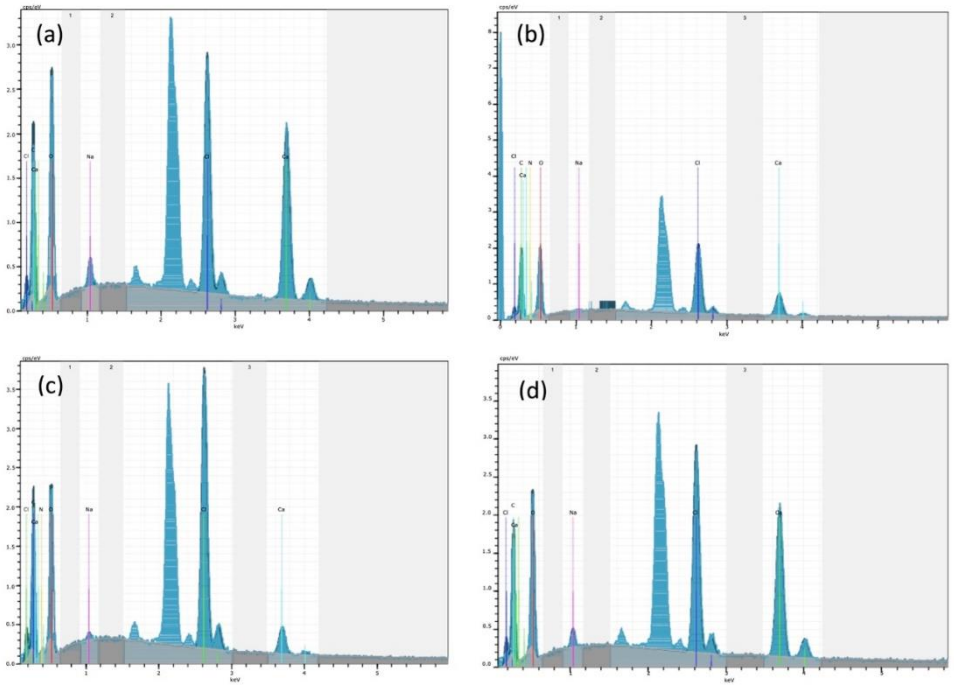

Figure 5. SEM-EDX profiles of vitamin D_2/D_3-exine-alginate-chitosan microcapsule system. (a) D_2-exine-alginate microcapsules, (b) D_2-exine-alginate-chitosan microcapsules, (c) D_3-exine-alginate microcapsules, (d) D_3-exine-alginate-chitosan microcapsules

Table 1. SEM-EDX results of D_2-exine-alginate microcapsules

Element	Mass [%]	Atomic [%]	Error [%]
Oxygen	65.92	65.41	21.3
Calcium	6.39	2.53	0.2
Chlorine	4.83	2.16	0.2
Carbon	22.37	29.56	7.9
Sodium	0.49	0.34	0.1

Table 2. SEM-EDX results of D_2-exine-alginate-chitosan microcapsules

Element	Mass [%]	Atomic [%]	Error [%]
Oxygen	65.77	62.16	21.2
Calcium	2.01	0.76	0.1
Chlorine	3.61	1.54	0.1
Carbon	26.07	32.83	9.0
Sodium	0.06	0.04	0.0
Nitrogen	2.47	2.67	1.8

Table 3. SEM-EDX results of D_3-exine-alginate microcapsules

Element	Mass [%]	Atomic [%]	Error [%]
Oxygen	65.28	64.48	21.3
Calcium	5.98	2.36	0.2
Chlorine	5.07	2.26	0.2
Carbon	23.28	30.63	8.4
Sodium	0.39	0.27	0.1

Table 4. SEM-EDX results of D_3-exine-alginate-chitosan microcapsules

Element	Mass [%]	Atomic [%]	Error [%]
Oxygen	63.80	60.82	20.6
Calcium	1.10	0.42	0.1
Chlorine	6.12	2.63	0.2
Carbon	25.56	32.46	8.9
Sodium	0.13	0.09	0.0
Nitrogen	3.29	3.58	2.2

3.2. Loading of Vitamin D Molecules into The Exine-Alginate-Chitosan Microcapsule System

The loading efficiencies of vitamin D_2 and D_3 into exine microcapsules were calculated based on mass measurements. The average weight of vitamin D_2-loaded exine microcapsules was 131.5 mg, while that of vitamin D_3 was 116.0 mg. It was concluded that 40.0 mg of vitamins D_2 and D_3 were initially loaded into 100 mg of microcapsules, resulting in loading efficiencies of 31.5 mg and 16.0 mg, respectively.

The process involved loading vitamins into the microcapsules of the exine, which were then coated with alginate. The surface of the microcapsules was analysed under a scanning electron microscope (SEM) at 100x, 350x, and 1000x dimensions (Figure 6). The study revealed that the exine particles loaded with vitamin D_2 and D_3 were embedded in the alginate matrix, and the exine capsules showed a non-uniform distribution on the microsphere. Additionally, the exine-alginate microcapsules were almost spherical in shape, and their dimensions were similar to each other. The dimensions of the D_2-exine-alginate spheres and D_3-exine-alginate spheres were approximately 1.3 mm (Figures 6 b–e).

Further analysis revealed the presence of cracks on the surfaces of the microcapsules at high magnifications (Figures 6 c–f). It was determined that the cracks were formed during the drying phase. As a result, the release properties of the microcapsules may not be predictable when D_2 or D_3-loaded exine microcapsules are used only with an alginate matrix, limiting their use as a vitamin supplement.

In the study, alginate microcapsules were prepared to contain vitamin D supplements. These microcapsules were then coated with chitosan, as shown in Figure 7. The chitosan-coated microcapsules were spherical in shape, with similar sizes and a thin chitosan layer. The microcapsules' size was approximately 1.3 mm, which did not change much after the coating process. During the drying phase, excess chitosan leaked from the microcapsules spread on the ground and dried there, as shown in Figures 7 b and e. The chitosan coating effectively closed the cracks on the microcapsules' surface, as shown in Figures 7 c and f. However, the vitamin D-loaded exine microcapsules on the surface were still visible even after the chitosan coating.

3.3. Vitamin D Release Profiles of The Exine-Alginate-Chitosan Microcapsule System

The release of vitamins D_2 and D_3 from microcapsules was studied over a period of time (30, 60, 120, and 240 min.) at 37°C. Table 5 presents the time-dependent release percentages calculated from the absorbance values. The results showed that the longer the duration of both vitamins, the higher the release rate.

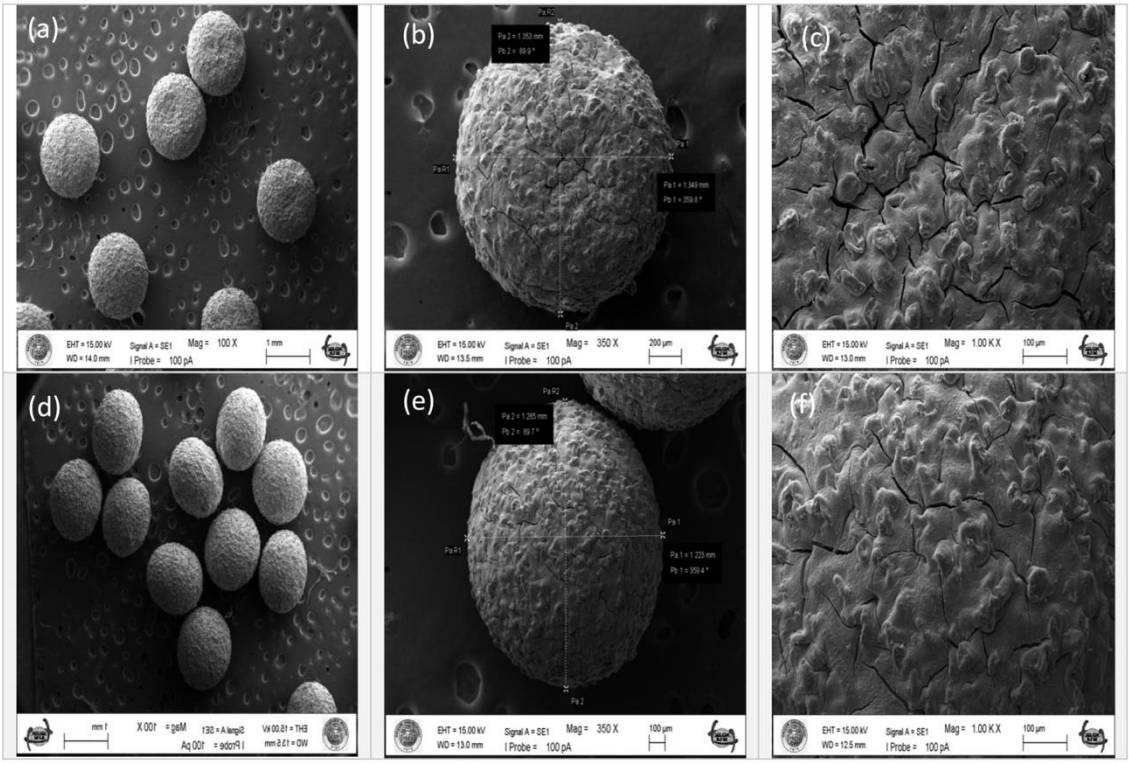

Figure 6. SEM images of the vitamin D-exine-alginate microcapsule system; (a-c): D_2 and (d-e): D_3

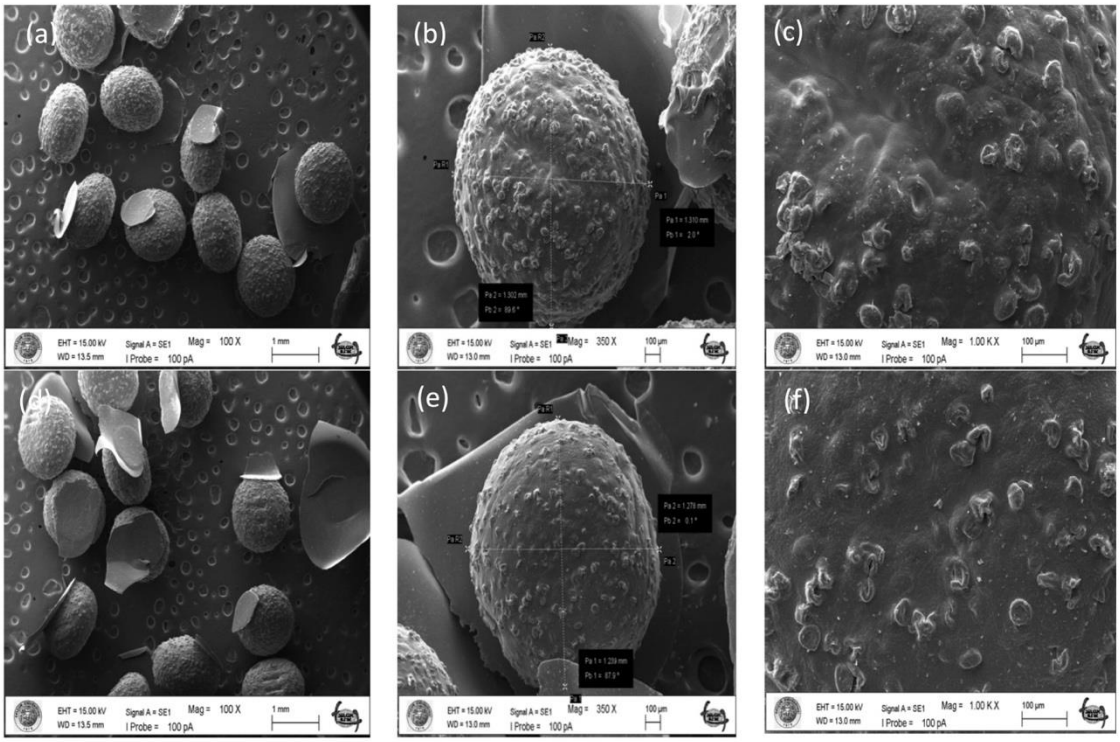

Figure 7. SEM images of the vitamin D-exine-alginate-chitosan microcapsule system; (a-c): D_2 and (d-e): D_3

Table 5. Time-dependent D_2 and D_3 release rates of the exine-alginate-chitosan microcapsule system

Time [min.]	D_2 release [%]	D_3 release [%]
30	2.6	1.8
60	3.1	2.3
120	4.3	4.4
240	4.9	5.0

The study examined the release of vitamins at different temperatures (4, 20, and 37°C) over a period of 120 minutes (Table 6). The results indicated that the highest release occurred at 37°C for both vitamins.

During the study, D_2 and D_3 vitamins were loaded into exine microcapsules, which were then distributed and encapsulated in an alginate matrix. The surface of the microcapsules was coated with chitosan, a biopolymer that dissolves easily in aqueous media, especially at low pH. However, since the release studies were conducted in alcohol, the chitosan coating and alginate matrix remained undissolved, preventing the solvent from reaching the microcapsules and releasing the vitamins. As a result, low release rates were observed for both vitamins. In a previous study, it was found that the release rate of vitamin D_2 from chitosan/ethylcellulose complex microcapsules was influenced by the chitosan coating layer, which caused a delay in its release [15]. The resistance of drug diffusion increased with an increase in the coating layer. The release of microcapsules in artificial gastric juice was very limited, indicating that the coating of vitamin D_2 could effectively delay drug release in gastric juice.

Table 6. Temperature-dependent D_2 and D_3 release rates of the exine-alginate-chitosan microcapsule system (in 120 min.)

Temperature [°C]	D_2 release [%]	D_3 release [%]
4	3.2	0.8
20	4.0	0.9
37	4.3	4.4

4. CONCLUSIONS

Herein the presented study demonstrated that it is possible to obtain sporopollenin exine microcapsules from *C. libani* pollens through a chemical process that involves the use of a microwave. This method is significantly faster and more energy efficient than traditional methods that use external heat sources. The structural integrity of the exine microcapsules remains intact even after undergoing microwave treatment. In this study, vitamins D_2 and D_3 were successfully loaded into the exine microcapsules using an ethanol medium. Alginate was found to be a suitable matrix for encapsulating the loaded exine microcapsules, and chitosan was used to coat them, thus increasing their structural stability. The study also revealed that the release of vitamins D_2 or D_3 from the exine-alginate-chitosan microcapsule system was dependent on both temperature and time. Further studies are required to evaluate the performance of vitamin D_2 or D_3-the exine microcapsule-alginate-chitosan microcapsule system in *in vitro* and *in vivo* studies.

Declaration of Ethical Standards

No ethical committee approval was required for the materials and methods used in the study.

Credit Authorship Contribution Statement

GD: Benchwork, experimental design, and data collection. IS: Conceptualization, study design, analysis and interpretation of data, manuscript writing, funding acquisition, and work supervision.

Declaration of Competing Interest

The authors state no financial or personal conflicts of interest.

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Data Availability

Data will be made available on request.

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